INSTRUCTION MANUAL

NEBNext® Multiplex Oligos for Enzymatic Methyl-seq (Unique Dual Index Primer Pairs)

NEB #E7140S/L

24/96 reactions

Version 4.0_6/24

Table of Contents

The Oligos Kit Includes:

The volumes provided are sufficient for preparation of up to 24 reactions (NEB #E7140S) and 96 reactions (NEB #E7140L). All reagents should be stored at –20°C. Colored bullets represent the color of the cap of the tube containing the reagent.

NEB #E7140S – NEBNext Multiplex Oligos for EM-seq™ (Unique Dual Index Primer Pairs):

- (red) NEBNext EM-seq Adaptor
- (blue) EM-seq Index Primer 1
- (blue) EM-seq Index Primer 2
- (blue) EM-seq Index Primer 3
- (blue) EM-seq Index Primer 4
- (blue) EM-seq Index Primer 5
- (blue) EM-seq Index Primer 6
- (blue) EM-seq Index Primer 7
- (blue) EM-seq Index Primer 8
- (blue) EM-seq Index Primer 9
- (blue) EM-seq Index Primer 10
- (blue) EM-seq Index Primer 11
- (blue) EM-seq Index Primer 12
- (blue) EM-seq Index Primer 13
- (blue) EM-seq Index Primer 14
- (blue) EM-seq Index Primer 15
- (blue) EM-seq Index Primer 16
- (blue) EM-seq Index Primer 17
- (blue) EM-seq Index Primer 18
- (blue) EM-seq Index Primer 19
- (blue) EM-seq Index Primer 20
- (blue) EM-seq Index Primer 21
- (blue) EM-seq Index Primer 22
- (blue) EM-seq Index Primer 23
- (blue) EM-seq Index Primer 24

NEB #E7140L – NEBNext Multiplex Oligos for EM-seq (Unique Dual Index Primer Pairs):

- (red) NEBNext EM-seq Adaptor

NEBNext Multiplex Oligos for EM-seq (Unique Dual Index Primer Pairs) 96 well plate

For the list of additional materials required, please check the manual for the NEBNext Enzymatic Methyl-seq Library Prep Kit.

Applications

The NEBNext Multiplex Oligos for Enzymatic Methyl-seq contains unique dual index primers and EM-seq adaptors that are needed to make EM-seq libraries. The oligos in this kit have been optimized for use with the NEBNext Ultra II DNA Library Prep Kit (NEB #E7645. except the included polymerase), Enzymatic Methyl-seq Conversion Module (NEB #E7125) and Q5U™ Master Mix (NEB #M0597).

Each EM-seq adaptor and primer must pass rigorous quality control standards, and for each new lot the entire set of reagents is functionally validated together with #E7645, #E7125 and #M0597 by construction of indexed libraries and sequenced on an Illumina sequencing platform.

Where larger volumes, customized or bulk packaging are required, we encourage consultation with the Customized Solutions team at NEB. Please complete the NEB Custom Contact Form at www.neb.com/CustomContactForm to learn more.

Workflow Overview

The EM-seq adaptor enables high-efficiency adaptor ligation and high library yields of Enzymatic Methyl-seq libraries. The NEBNext EM-seq adaptor ligates to end-repaired, dA-tailed DNA.

During PCR, indexes are incorporated using the Unique Dual Index Primer Pairs provided in this kit. NEBNext Q5U Master Mix uses a modified Q5® polymerase that can amplify uracil containing templates (NEB #M0597). The Unique Dual Index Primers enable multiplexing and have been designed to address index hopping. The 24 reaction kit is supplied with 24 individual single use tubes with premixed i5 and i7, 8-base index primers. For the 96 reaction kit, each well of a 96 well plate contains an i5 and i7 primer pair. Both primers contain an 8-base index and are supplied with a pierceable foil seal for easy, single use. The index sequences from the 24-reaction kit (NEB #E7140S) and the 96-reaction kit (NEB #E7140L) are compatible, and can be combined to multiplex up to 120 reactions on some Illumina Sequencing Instruments.

Please refer to the Enzymatic Methyl-seq Kit Manual (NEB #E7120) for using the NEBNext Multiplex Oligos for Enzymatic Methyl-seq.

NEBNext EM-seq Adaptor Trimming

The following sequences are used for adaptor trimming of NEBNext adaptors for Illumina:

Read 1 AGATCGGAAGAGCACACGTCTGAACTCCAGTCA

Read 2 AGATCGGAAGAGCGTCGTGTAGGGAAAGAGTGT

Index Sequence Files

For a link to download a sample sheet with the index sequences for use with the Illumina Experiment Manager (IEM) please go to our FAQs or Usage Guidelines tab on the E7140 product page on www.neb.com:

NEBNext Multiplex Oligos for Enzymatic Methyl-seq (Unique Dual Index Primer Pairs)

Section 1
Setting up the PCR Reactions

Symbols

This caution sign signifies a step in the protocol that has two paths leading to the same end point, like the number of samples to be processed.

For NEB #E7140S, follow the protocol in Section 1.1. For NEB #E7140L, follow the protocol in Section 1.2.

1.1. Setting up the PCR reactions (24 Reaction kit, NEB #E7140S)

1.1.1. Determine the number of libraries that will be amplified and pooled for subsequent sequencing.

1.1.2. Ensure that a valid combination of index primers is chosen based on color balance guidelines in Section 2A.

1.1.3. Thaw the EM-seq Index Primer tubes for 10 minutes at room temperature.

1.1.4. Briefly centrifuge primers supplied in individual tubes to collect all of the primer at the bottom of each tube.

1.1.5. Remove the appropriate amount of primer from each single use tube using individual pipette tips for each primer. Each tube contains enough primer mix for one reaction.

1.1.6. Proceed with the PCR reaction according to the Enzymatic Methyl-seq manual (NEB #E7120).

1.2. Setting up the PCR reactions (96 Reaction kit, NEB #E7140L)

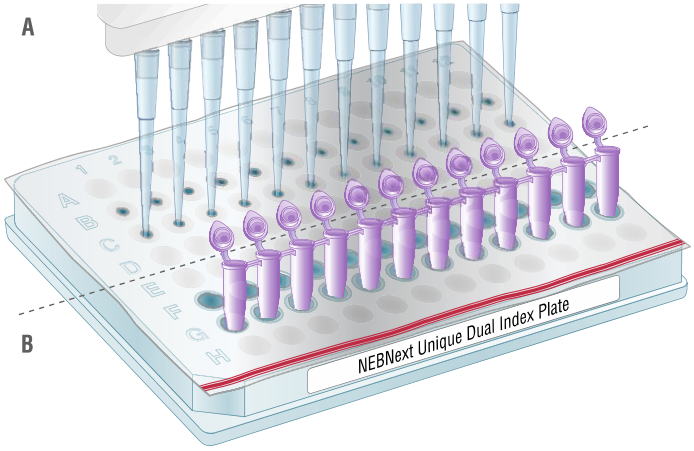
1.2.1. Determine the number of libraries that will be amplified and pooled for subsequent sequencing.

1.2.2. Ensure that a valid combination of index primers is chosen based on color balance guidelines in Section 2B.

1.2.3. Thaw the NEBNext 96 Unique Dual Index Pairs Plate for 10 minutes at room temperature.

1.2.4. Remove the hard-plastic plate cover from the 96 well plate. Briefly centrifuge the plate (280 x g for ~1 min) to collect all of the primer at the bottom of each well.

Figure 1.1. NEBNext 96 Unique Dual Index Pairs Plate



1.2.5. Orient the NEBNext Unique Dual Index primer plate as indicated in Figure 1.1 (red stripe towards the user). With a pipette tip, pierce the desired well(s) (Figure 1.1A) and transfer the volume of primer mix required for the PCR reaction to the PCR plate/tubes. It is important to change pipette tips before piercing a new well to avoid cross contamination of indexed primers. Alternatively, the wells can be pierced using the bottom of clean PCR strip tubes (see Figure 1.1B) prior to pipetting the primer mix. Use a new, clean strip tube for each new well to be pierced.

Note: Each well contains the NEBNext Unique Dual Index primer pair. There is enough primer in each well for one PCR reaction. Do not reuse primer if the seal has been previously pierced to avoid contamination with other indexed primers.

1.2.6. Proceed with the PCR reaction according to the Enzymatic Methyl-seq manual (NEB #E7120).

Section 2
Index Pooling Guidelines

For a link to download a sample sheet with the index sequences for use with the Illumina Experiment Manager (IEM) please go to our FAQ's tab on www.neb.com/E7140 – NEBNext Multiplex Oligos for Enzymatic Methyl-seq (Unique Dual Index Primer Pairs) (NEB #E7140).

For all HiSeq®/MiSeq® sequencers:

Illumina uses four channel chemistry with a red laser/LED to sequence bases A and C and a green laser/LED to sequence bases G and T. For each cycle, both the red and the green channel need to be read to ensure proper image registration (i.e. A or C must be in each cycle, and G or T must be in each cycle). If this color balance is not maintained, sequencing the index read could fail. The following tables list some valid combinations (up to 8-plex) for each Set that can be sequenced together. For combinations > 8 choose any column and add any plex combinations as needed.

For the NovaSeq®6000/ NextSeq®/MiniSeq®:

Utilize red/green or blue/green 2 color chemistry, valid index combinations must include some indices that do not start with GG in the first two cycles. See Illumina document Document # 1000000041074 v12

For the NovaSeq®X and X Plus:

Utilize blue/ green 2 color chemistry, valid index combinations must include some indices that do not start with GG in the first two cycles For additional NovaSeq X and X Plus color balancing guidelines please contact NEB technical support at info@neb.com

Low Plex pooling options shown here are only for Illumina four channel chemistry.

Four Channel Chemistry Color Balancing

***Forward Strand Workflow** for the following instruments: NovaSeq 6000 with v1.0 reagents kits, MiniSeq with rapid reagent kits, MiSeq®, HiSeq® 2000/2500 (paired-end flow cell), HiSeq 3000/4000 (single-read flow cell).

***Reverse Complement Workflow** for the following instruments: iSeq 100, MiniSeq with standard reagent kits, NextSeq Systems, NovaSeq 6000 with v1.5 reagent kits, HiSeq 2000/5000 (single-read flow cell), HiSeq 3000/4000 (paired-end flow cell).

See Illumina Document "Indexed Sequencing Overview" 15057455 and Illumina Guidelines for reverse complementing i5 sequences" for demultiplexing Illumina Knowledge Article #1800 Guidelines for reverse complementing i5 sequences for demultiplexing - Illumina Knowledge.

2A. Index Pooling Guidelines: 24-Reaction Kit

NEBNext Multiplex Oligos for Enzymatic Methyl-seq (**NEB #E7140S**). Use Table 2.1 for some suggested combinations.

Table 2.1.

PLEX	INDEX NUMBER
2	1 and 2 3 and 4 5 and 6 7 and 8
≥ 3	Any 2 plex plus any other index

The index primer sequences, for different Illumina sequencer input sheets are indicated in Table 2.2.

Table 2.2 Index Sequences (Color coded based on four channel red/green guidelines)

INDEX NUMBER	EXPECTED i7 INDEX READ	EXPECTED i5 INDEX READ	
		FORWARD STRAND WORKFLOW*	REVERSE COMPLEMENT WORKFLOW*
1	CACTGTAG	AAGCGACT	AGTCGCTT
2	GTGCACGA	TGATAGGC	GCCTATCA
3	AAGCGACT	ACGAATCC	GGATTCGT
4	TGATAGGC	GTCTGAGT	ACTCAGAC
5	ACGAATCC	ATTACCCA	TGGGTAAT
6	GTCTGAGT	GACTTGTG	CACAAGTC
7	ATTACCCA	CACTGTAG	CTACAGTG
8	GACTTGTG	GTGCACGA	TCGTGCAC
9	TTCAATAG	TCCCACGA	TCGTGGGA
10	GTTTGCTC	ACCAACAG	CTGTTGGT
11	ACCGGAGT	AAGGAAGG	CCTTCCTT
12	CTTGACGA	GCACACAA	TTGTGTGC
13	TGTTCGCC	AGGTAGGA	TCCTACCT
14	ACAAGGCA	TCGCGCAA	TTGCGCGA
15	CCTGTCAA	ATGGCTGT	ACAGCCAT
16	CCATCCGC	AAGGCGTA	TACGCCTT
17	ATGGCTGT	CCTGTCAA	TTGACAGG
18	AAGGCGTA	CCATCCGC	GCGGATGG
19	AGGTAGGA	TGTTCGCC	GGCGAACA
20	TCGCGCAA	ACAAGGCA	TGCCTTGT
21	AAGGAAGG	ACCGGAGT	ACTCCGGT
22	GCACACAA	CTTGACGA	TCGTCAAG
23	TCCCACGA	TTCAATAG	CTATTGAA
24	ACCAACAG	GTTTGCTC	GAGCAAAC

2B. Index Pooling Guidelines: 96-Reaction Kit

NEBNext Multiplex Oligos for Enzymatic Methyl-seq (**NEB #E7140L**). Use Table 2.3 for some suggested combinations.

Table 2.3.

PLEX	WELL POSITION
< 4	Not recommended
4	A6, B6, C6, and D6 A12, B12, C12, and D12 B6, C6, D6, and E6 B12, C12, D12, and E12 C1, D1, E1, and F1 C7, D7, E7, and F7 E4, F4, G4, and H4 E10, F10, G10, H10
5	A1, B1, C1, D1, E1 A6, B6, C6, D6, E6 A7, B7, C7, D7, E7 A12, B12, C12, D12, E12 B1, C1, D1, E1, F1 B6, C6, D6, E6, F6 B7, C7, D7, E7, F7 B12, C12, D12, E12, F12 C1, D1, E1, F1, G1 C2, D2, E2, F2, G2 C4, D4, E4, F4, G4 C7, D7, E7, F7, G7 C8, D8, E8, F8, G8 C10, D10, E10, F10, G10 D4, E4, F4, G4, H4 D10, E10, F10, G10, H10
6-7	Any 5 plex plus 1-2 adjacent wells from the same column
8	Any column

Table 2.4. lists each index sequence color coded to correspond to the four color chemistry red/green channel. For combinations of valid indices, ensure that you will have signals in both the red and green channels in each cycle. See below for examples of Good and Bad index combinations based on four color chemistry guidelines:

BAD																									
WELL POSITION	EXPECTED i7 INDEX READ								EXPECTED i5 INDEX READ																
									FORWARD STRAND WORKFLOW*								REVERSE COMPLEMENT WORKFLOW*								
E8	T	A	T	G	G	C	A	C	T	T	G	C	G	A	G	A	T	C	T	C	G	C	A	A
F8	G	A	A	T	C	A	C	C	G	A	A	C	G	A	A	G	C	T	T	C	G	T	T	C
G8	G	T	A	A	G	G	T	G	C	G	A	A	T	T	G	C	G	C	A	A	T	T	C	G
H8	C	G	A	G	A	G	A	A	G	G	A	A	G	A	G	A	T	C	T	C	T	T	C	C
	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	X	X	✓	✓	✓	✓	✓	✓	X	X	✓	✓	✓
A1	T	T	A	C	C	G	A	C	C	G	A	A	T	A	C	G	C	G	T	A	T	T	G	G
B1	T	C	G	T	C	T	G	A	G	T	C	C	T	T	G	A	T	C	A	A	G	G	A	C
C1	T	T	C	C	A	G	G	T	C	A	G	T	G	C	T	T	A	A	G	C	A	C	T	G
D1	T	A	C	G	G	T	C	T	T	C	C	A	T	T	G	C	G	C	A	A	T	G	G	A
	X	✓	✓	✓	✓	X	✓	✓	✓	✓	✓	✓	X	✓	✓	✓	✓	✓	✓	X	✓	✓	✓	✓

GOOD																									
WELL POSITION	EXPECTED i7 INDEX READ								EXPECTED i5 INDEX READ																
									FORWARD STRAND WORKFLOW*								REVERSE COMPLEMENT WORKFLOW*								
C1	T	T	C	C	A	G	G	T	C	A	G	T	G	C	T	T	A	A	G	C	A	C	G	G
D1	T	A	C	G	G	T	C	T	T	C	C	A	T	T	G	C	G	C	A	A	T	G	G	A
E1	A	A	G	A	C	C	G	T	G	T	C	G	A	T	T	G	C	A	A	T	C	G	A	C
F1	C	A	G	G	T	T	C	A	A	T	A	A	C	G	C	C	G	G	C	G	T	T	A	T
	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
A12	C	G	G	C	A	T	T	A	G	T	C	A	G	T	C	A	T	G	A	C	T	G	C	C
B12	C	A	C	G	C	A	A	T	C	C	T	T	C	C	A	T	A	T	G	G	A	A	G	G
C12	G	G	A	A	T	G	T	C	A	G	G	A	A	C	A	C	G	T	G	T	T	C	C	T
D12	T	G	G	T	G	A	A	G	C	T	T	A	C	A	G	C	G	C	T	G	T	A	A	G
	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓

The index primer sequences, for different Illumina sequencer input sheets are indicated in Table 2.5. (For a link to download a sample sheet with the index sequences for use with the Illumina Experiment Manager (IEM) please go to our FAQs or Usage Guidelines tab on the NEB #E7140 product page on www.neb.com: NEBNext Multiplex Oligos for Enzymatic Methyl-seq (Unique Dual Index Primer Pairs).

Table 2.5 Index Sequences (Color coded based on based on four channel red/ green guidelines)

WELL POSITION	EXPECTED i7 INDEX READ	EXPECTED i5 INDEX READ	
		FORWARD STRAND WORKFLOW*	REVERSE COMPLEMENT WORKFLOW*
A1	TTACCGAC	CGAATACG	CGTATTCG
B1	TCGTCTGA	GTCCTTGA	TCAAGGAC
C1	TTCCAGGT	CAGTGCTT	AAGCACTG
D1	TACGGTCT	TCCATTGC	GCAATGGA
E1	AAGACCGT	GTCGATTG	CAATCGAC
F1	CAGGTTCA	ATAACGCC	GGCGTTAT
G1	TAGGAGCT	GCCTTAAC	GTTAAGGC
H1	TACTCCAG	GGTATAGG	CCTATACC
A2	AGTGACCT	TCTAGGAG	CTCCTAGA
B2	AGCCTATC	TGCGTAAC	GTTACGCA
C2	TCATCTCC	CTTGCTAG	CTAGCAAG
D2	CCAGTATC	AGCGAGAT	ATCTCGCT
E2	TTGCGAGA	TATGGCAC	GTGCCATA
F2	GAACGAAG	GAATCACC	GGTGATTC
G2	CGAATTGC	GTAAGGTG	CACCTTAC
H2	GGAAGAGA	CGAGAGAA	TTCTCTCG
A3	TCGGATTC	CGCAACTA	TAGTTGCG
B3	CTGTACCA	CACAGACT	AGTCTGTG
C3	GAGAGTAC	TGGAAGCA	TGCTTCCA
D3	TCTACGCA	CAATAGCC	GGCTATTG
E3	GCAATTCC	CTCGAACA	TGTTCGAG
F3	CTCAGAAG	GGCAAGTT	AACTTGCC
G3	GTCCTAAG	AGCTACCA	TGGTAGCT
H3	GCGTTAGA	CAGCATAC	GTATGCTG
A4	CAAGGTAC	CGTATCTC	GAGATACG
B4	AGACCTTG	TTACGTGC	GCACGTAA
C4	GTCGTTAC	AGCTAAGC	GCTTAGCT
D4	GTAACCGA	AAGACACC	GGTGTCTT
E4	GAATCCGT	CAACTCCA	TGGAGTTG
F4	CATGAGCA	GATCTTGC	GCAAGATC
G4	CTTAGGAC	CTTCACTG	CAGTGAAG
H4	ATCTGACC	CTCGACTT	AAGTCGAG
A5	TCCTCATG	GTACACCT	AGGTGTAC
B5	AGGATAGC	CCAAGGTT	AACCTTGG
C5	GGAGGAAT	GAACGGTT	AACCGTTC
D5	GACGTCAT	CCAGTTGA	TCAACTGG
E5	CCGCTTAA	GTCATCGT	ACGATGAC
F5	GACGAACT	CAATGCGA	TCGCATTG
G5	TCCACGTT	GGTTGAAC	GTTCAACC
H5	AACCAGAG	CTTCGGTT	AACCGAAG

WELL POSITION	EXPECTED i7 INDEX READ	EXPECTED i5 INDEX READ	
		FORWARD STRAND WORKFLOW*	REVERSE COMPLEMENT WORKFLOW*
A6	GTCAGTCA	CGGCATTA	TAATGCCG
B6	CCTTCCAT	CACGCAAT	ATTGCGTG
C6	AGGAACAC	GGAATGTC	GACATTCC
D6	CTTACAGC	TGGTGAAG	CTTCACCA
E6	TACCTGCA	GGACATCA	TGATGTCC
F6	AGACGCTA	GGTGTACA	TGTACACC
G6	CAACACAG	GATAGCCA	TGGCTATC
H6	GTACCACA	CCACAACA	TGTTGTGG
A7	CGAATACG	TTACCGAC	GTCGGTAA
B7	GTCCTTGA	TCGTCTGA	TCAGACGA
C7	CAGTGCTT	TTCCAGGT	ACCTGGAA
D7	TCCATTGC	TACGGTCT	AGACCGTA
E7	GTCGATTG	AAGACCGT	ACGGTCTT
F7	ATAACGCC	CAGGTTCA	TGAACCTG
G7	GCCTTAAC	TAGGAGCT	AGCTCCTA
H7	GGTATAGG	TACTCCAG	CTGGAGTA
A8	TCTAGGAG	AGTGACCT	AGGTCACT
B8	TGCGTAAC	AGCCTATC	GATAGGCT
C8	CTTGCTAG	TCATCTCC	GGAGATGA
D8	AGCGAGAT	CCAGTATC	GATACTGG
E8	TATGGCAC	TTGCGAGA	TCTCGCAA
F8	GAATCACC	GAACGAAG	CTTCGTTC
G8	GTAAGGTG	CGAATTGC	GCAATTCG
H8	CGAGAGAA	GGAAGAGA	TCTCTTCC
A9	CGCAACTA	TCGGATTC	GAATCCGA
B9	CACAGACT	CTGTACCA	TGGTACAG
C9	TGGAAGCA	GAGAGTAC	GTACTCTC
D9	CAATAGCC	TCTACGCA	TGCGTAGA
E9	CTCGAACA	GCAATTCC	GGAATTGC
F9	GGCAAGTT	CTCAGAAG	CTTCTGAG
G9	AGCTACCA	GTCCTAAG	CTTAGGAC
H9	CAGCATAC	GCGTTAGA	TCTAACGC
A10	CGTATCTC	CAAGGTAC	GTACCTTG
B10	TTACGTGC	AGACCTTG	CAAGGTCT
C10	AGCTAAGC	GTCGTTAC	GTAACGAC
D10	AAGACACC	GTAACCGA	TCGGTTAC
E10	CAACTCCA	GAATCCGT	ACGGATTC
F10	GATCTTGC	CATGAGCA	TGCTCATG
G10	CTTCACTG	CTTAGGAC	GTCCTAAG
H10	CTCGACTT	ATCTGACC	GGTCAGAT

WELL POSITION	EXPECTED i7 INDEX READ	EXPECTED i5 INDEX READ	
		FORWARD STRAND WORKFLOW*	REVERSE COMPLEMENT WORKFLOW*
A11	GTACACCT	TCCTCATG	CATGAGGA
B11	CCAAGGTT	AGGATAGC	GCTATCCT
C11	GAACGGTT	GGAGGAAT	ATTCCTCC
D11	CCAGTTGA	GACGTCAT	ATGACGTC
E11	GTCATCGT	CCGCTTAA	TTAAGCGG
F11	CAATGCGA	GACGAACT	AGTTCGTC
G11	GGTTGAAC	TCCACGTT	AACGTGGA
H11	CTTCGGTT	AACCAGAG	CTCTGGTT
A12	CGGCATTA	GTCAGTCA	TGACTGAC
B12	CACGCAAT	CCTTCCAT	ATGGAAGG
C12	GGAATGTC	AGGAACAC	GTGTTCCT
D12	TGGTGAAG	CTTACAGC	GCTGTAAG
E12	GGACATCA	TACCTGCA	TGCAGGTA
F12	GGTGTACA	AGACGCTA	TAGCGTCT
G12	GATAGCCA	CAACACAG	CTGTGTTG
H12	CCACAACA	GTACCACA	TGTGGTAC

Kit Components

NEB #E7140S Table of Components

NEB #	PRODUCT NAME	VOLUME
E7165A	NEBNext Adaptor for EM-seq	0.06 ml
E7141A	EM-seq Index Primer 1	0.005 ml
E7142A	EM-seq Index Primer 2	0.005 ml
E7143A	EM-seq Index Primer 3	0.005 ml
E7144A	EM-seq Index Primer 4	0.005 ml
E7145A	EM-seq Index Primer 5	0.005 ml
E7146A	EM-seq Index Primer 6	0.005 ml
E7147A	EM-seq Index Primer 7	0.005 ml
E7148A	EM-seq Index Primer 8	0.005 ml
E7149A	EM-seq Index Primer 9	0.005 ml
E7150A	EM-seq Index Primer 10	0.005 ml
E7151A	EM-seq Index Primer 11	0.005 ml
E7152A	EM-seq Index Primer 12	0.005 ml
E7153A	EM-seq Index Primer 13	0.005 ml
E7154A	EM-seq Index Primer 14	0.005 ml
E7155A	EM-seq Index Primer 15	0.005 ml
E7156A	EM-seq Index Primer 16	0.005 ml
E7157A	EM-seq Index Primer 17	0.005 ml
E7158A	EM-seq Index Primer 18	0.005 ml
E7159A	EM-seq Index Primer 19	0.005 ml
E7160A	EM-seq Index Primer 20	0.005 ml
E7161A	EM-seq Index Primer 21	0.005 ml
E7162A	EM-seq Index Primer 22	0.005 ml
E7163A	EM-seq Index Primer 23	0.005 ml
E7164A	EM-seq Index Primer 24	0.005 ml

NEB #E7140L Table of Components

NEB#	PRODUCT NAME	VOLUME
E7165AA	NEBNext Adaptor for EM-seq	0.24 ml
E7166A	NEBNext 96 Unique Dual Index Primer Pairs Plate	1 plate (5 µl/well)

Revision History

REVISION #	DESCRIPTION	DATE
1.0	N/A	2/19
2.0	Applied new manual format.	2/20
3.0	Updated tables to have the most current Illumina instrument information and removed HiSeqX.	2/21
4.0	Updated color balancing guidelines. Added sections on adaptors and on index sequence files. Also added new logo to header and footer and updated legal footnote.	6/24

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